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13. ABSTRACT (Maximum 200 words)
Depleted uranium (DU)-containing shrapnel was found in the wounds of several Gulf War veterans. DU may be more hazardous than other shrapnel because of its radioactivity and known toxicity to the kidney. Predictions of risk are necessary to guide the medical management of soldiers with DU-bearing wounds both now and in the future. We are determining the carcinogenicity of radioactive DU fragments in tissues relative to nonradioactive foreign-body fragments and assessing the potential for renal toxicity of DU fragments by correlating urine and kidney concentrations of U with time after implantation. DU fragments of differing sizes and shapes have been implanted in the soft tissue of rats to compare with results from animals implanted with inert metals. In this way a toxicity ratio will be determined that can be used to predict the expected response in humans from the known response of humans to relatively inert shrapnel. A pilot study was used to determine important experimental design parameters for studying the foreign-body response using this test system in animals. Parameters defined include fragment *in vitro* and *in vivo* solubility, optimal fragment size and shape for implantation, changes in the surface characteristics of fragments that could be important in carcinogenesis, and determination that fragments of DU alloyed with titanium DU(Ti) would be more desirable than nonalloyed DU based on particle solubility. A long-term carcinogenesis study of DU fragments implanted in the muscles of rats is in progress.

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I. INTRODUCTION

A. Purpose and Scope of Work

The purpose of this work is to determine the relative carcinogenicity of depleted uranium (DU) fragments embedded in soft tissues and the renal toxicity from chronic exposure to systemic uranium (U). These determinations will be made from the study of rats exposed to embedded DU. The results of the studies in rats will be used to estimate the carcinogenicity and renal toxicity of DU embedded in humans.

Two hypotheses are being tested:

Depleted U-0.75% Ti alloy [DU(Ti)] is more carcinogenic in muscle tissues than tantalum (Ta) metal. We theorize that the chronic low-level irradiation of tissues surrounding embedded DU(Ti) fragments will increase the carcinogenic potency of the metal fragments. The objective of testing this hypothesis is to determine the relative risk of radioactive fragments relative to nonradioactive fragments so that informed judgements can be made about the clinical management of veterans with DU(Ti) fragments embedded in their soft tissues.

The specific aim of this study is to determine experimentally the relative carcinogenicity of radioactive DU(Ti) and a nonradioactive inert metal, Ta. This relative carcinogenicity in rats will be used in a ratio so that the carcinogenicity of DU(Ti) in humans can be estimated using the following relationship:

$$\{\text{DU(Ti) toxicity/inert metal toxicity}\}_{\text{rat}} = \{\text{DU(Ti) toxicity/inert metal toxicity}\}_{\text{human}}$$

This approach is similar to the toxicity ratio method previously described to compare the risk of radiation-induced bone cancer in dogs and humans (Mays *et al.*, 1986) and mice and humans (Finkel and Biskis, 1968). The information for inert metal toxicity in humans arises from foreign-body carcinogenesis data related to metals used for implants, stainless steel, shrapnel, etc. (Brand and Brand, 1980; Brand, 1994; Galante *et al.*, 1991). Ta metal is physiologically inert and has been used in metal implants in animals and humans for many years (Beliles *et al.*, 1994) without observed biologic effects. Thorotrast® is used as the positive control for radioactive material in rats. The responses in rats will be related to carcinogenic responses in humans exposed to Thorotrast® deposited in subcutaneous locations (Liebermann *et al.*, 1995).

It is well known that rodents are much more sensitive to foreign-body carcinogenesis than humans (Furst, 1981). Thus, the direct test of carcinogenicity is rigorous

and should not yield a false-negative result. On the other hand, a positive result cannot be extrapolated directly to the human situation, only the relative effect. Rats with surgical manipulations similar to those used to insert the implants are sham controls.

The incidence of subcutaneous tumors will be compared among dose groups by using a Cox proportional hazards (CPH) model. These types of models take into account not only the total incidence of tumors but also the times at which the tumors occur in order to obtain more power to test for statistically significant differences and to provide additional insight into the process of carcinogenesis. The relative risks estimated from the model for comparing DU to an inert metal in the rat are toxicity ratios of the two materials that will then be applied to humans using the observed foreign-body toxicity of inert metals such as Ta metal. Because of the limited data available on foreign-body carcinogenesis in humans (e.g., Brand and Brand, 1980), we can only define an upper limit for toxicity. Data from rats injected with Thorotrast® will also be analyzed with a CPH model to understand the role of radiation dose and provide another comparison with humans.

The second hypothesis: Urinary concentrations of U are directly correlated with the renal concentrations of U and will reach a steady state after intramuscular implantation of DU(Ti) fragments. The objective of testing this hypothesis is to determine if the renal concentration of U reaches a steady state and produces overt signs of toxicity. This will enable informed judgements to be made about clinical management of veterans who are excreting U in their urine from DU(Ti) fragments embedded in their tissues.

The specific aims of this study are to determine: 1) the time course to achieve a steady-state renal U concentration from an implanted DU source and 2) if toxicity is present at the steady-state concentration. In response to earlier reviews, this portion of the project is restricted to work that will obtain as much information as possible about the renal toxicity of U in the animals that have implanted DU fragments and are held for long periods. Accordingly, the scope of these studies on renal toxicity is limited.

B. Summary of Previous Work in Project

The initial approach to determining the carcinogenic potential of DU or DU(Ti) was based on the model of foreign-body carcinogenesis in mice as used by Brand *et al.* (1975). These investigators delineated the various etiologic factors and stages of foreign-body

carcinogenesis by studying reactions in the subcutis of mice implanted with foreign bodies having large, smooth surfaces.

It was necessary, however, to clarify a number of critical variables before using this model to study the carcinogenic potential of DU or DU(Ti). A pilot study addressed three of these critical variables by determining: 1) the *in vivo* solubility of DU during the first 60 d after its implantation in rats and mice, 2) the changes in the surface characteristics of the DU foil after implantation, and 3) histological responses of rats and mice to the implanted DU during this time. The experimental design is shown in Table 1. Two types of foils containing DU were used. One contained only DU, the other in which DU was alloyed with 0.75% Ti [DU(Ti)]. The foils measured approximately 20 mm × 15 mm × 1.5 mm. Ta foils of similar size were used as control implants. The composition of the DU(Ti) foils was the same as that used in a study at the Armed Forces Radiobiology Research Institute (AFFRI) on the dissolution of DU(Ti) pellets in rats (Castro *et al.*, 1996) and has been described in detail (Daxon, 1995).

Table 1

Experimental Design for the Study of Dissolution and Excretion of Uranium and Early Biological Effects of Subcutaneously Implanted DU, DU(Ti), or Ta Metal Foils in Male Rats and Mice

	Foil Type and Number of Animals Sacrificed at 30 d			Foil Type and Number of Animals Sacrificed at 60 d[a]			
Rodent	DU	DU(Ti)	Ta	DU	DU(Ti)	Ta	Total
F344 Rats[b]	5	5	4	5	5	4	28
CBA/J Mice[b]	5	5	4	5	5	4	28
Total	10	10	8	10	10	8	56

[a]Twenty-four-hour urine samples were collected from three rats and three mice on days –2, –1, 1, 2, 3, 4, 7, 14, 21, 28, 35, 42, 49, 56, and 60 before and after DU and DU(Ti) foils were implanted and on days –2, 7, 14, 28, and 35 before and after Ta foils were implanted.
[b]Deaths of animals prior to the scheduled sacrifice time are discussed in the text.

The results of the pilot study indicated that DU and DU(Ti) foils dissolved in the subcutis of both mice and rats. U concentrations in the kidneys increased throughout the 60-d observation period. The DU(Ti) foils dissolved more rapidly than DU. In addition, both types of DU foils broke down in the subcutis, becoming roughened and causing a moderate inflammatory

cell infiltration in the surrounding tissues. DU(Ti) foils caused more inflammation and more renal damage. Both of these effects most likely relate to the greater solubility of DU(Ti).

It was evident that the subcutaneous foreign-body carcinogenesis model system described by Brand *et al.* (1975) could not be applied to a study of the carcinogenesis of implanted foils containing DU. Key elements in the Brand foreign-body carcinogenesis model are a smooth surface on the foreign-body and a relative lack of inflammatory response. Therefore, results of the pilot study indicated that another approach must be taken to assess the carcinogenic potential of DU compounds.

II. BODY OF THE REPORT

A. *In vitro* Dissolution Study

The biokinetics and dosimetry of embedded DU fragments depend to a significant extent on the physicochemical properties of the DU *in vivo*. In particular, the distribution of both radiation and chemical doses to the assumed principal target organs, the wound site and the kidney, will be determined by the *in vivo* dissolution kinetics of U. The more insoluble the DU fragments, the greater the dose to the wound site, and the lesser to the kidney. Conversely, as solubility increases, the dose to the kidney increases. It is, therefore, important to measure the dissolution rate of DU *in vivo* and *in vitro*. The former provide data needed for biokinetic/ metabolic modeling, whereas the latter can provide insight into the mechanisms of dissolution of U present as metal fragments. These results of an *in vitro* dissolution study are designed to supplement the *in vivo* dissolution results being obtained in rats serially killed after intramuscularly implantation of DU fragments.

Two types of DU metal fragments were used in this study: 1) DU fragments cut to 1 cm × 1 cm size, 0.15 cm thick; and 2) DU containing 0.75% titanium DU(Ti) and cut to the same size as the DU fragments. Both materials were obtained from Manufacturing Sciences Corp. (Oak Ridge, TN) as foils, and were cut into the requisite sizes at Lovelace Respiratory Research Institute (LRRI) using a standard mechanical shear. Uranium blanks were obtained by using Ta fragments run in parallel *in vitro* dissolution systems.

The *in vitro* measurements were done by placing one of the DU or DU(Ti) fragments into a LRRI static dissolution cell (Kanapilly and Goh, 1973), which consists of a "sandwich" of two 47-mm hydrophilic polysulphone membrane filters (Tuffryn-HT, 0.2 μm pore size, Pall Gelman Laboratory, Ann Arbor, MI) and a screw-fastened Teflon ring that holds the

filters securely around the test material, the DU fragments. The static cell is then immersed in a volume of 50 mL of solvent, and maintained at room temperature without stirring. At selected times (4 h; 1, 4, 8, 11, 18, 22, 25, and 28 d), the solvent was exchanged with an equal volume of fresh solvent. At the termination of the dissolution study (28 d), the dissolution cell containing the undissolved U was removed from the solvent, disassembled, photographs taken, and the various parts of the dissolution system submitted for U analysis (see below). The two solvents used in this study were synthetic serum ultrafiltrate (SUF; Eidson and Griffith, 1984), and distilled, deionized water adjusted to pH 5.0 ±0.1 with dilute HCl. The composition of SUF is provided in Table 2. The pH of the SUF solutions was maintained at 7.4 ± 0.1 by maintaining an atmosphere of 5% CO_2 in air in the head space above each liquid sample. The pH was checked routinely throughout the course of the experiment. The pH 5 solvent was handled in a similar manner.

Table 2

Composition of Synthetic Serum Ultrafiltrate (SUF)

Solute	Molar Concentration
NaCl	0.116
NH_4Cl	0.010
$NaHCO_3$	0.027
Glycine	0.0050
$Na_3Citrate$	0.0002
$CaCl_2$	0.0002
L-Cysteine	0.001
H_2SO_4	0.0005
Na_2HPO_4	0.0012
DTPA[a]	0.0002
ABDAC[b]	50 ppm

[a]Diethylenetriaminepentaacetic acid, not present in blood serum; used to minimize binding of solubilized actinides to container and apparatus surfaces.

[b]Alkylbenzyldimethylammonium chloride added as an antibacterial, antifungal agent.

All samples were analyzed for U content using a kinetic phosphorescence analyzer (KPA-11, Chemchek Instruments, Inc.). This method measures the concentration of soluble U ions by laser excitation, and is a very sensitive method for measuring U concentration. The lower limit of detection is 50 parts per trillion. Aliquots of the solvent samples were taken as is and measured directly. The filter and filter holder samples were ashed at 550°C and dissolved in 1 M HNO_3. The remaining insoluble U fragment and associated particles were dissolved in concentrated nitric acid, then diluted to 1 M HNO_3 for aliquoting and U measurement.

During the course of the *in vitro* dissolution study, it was noted within 4 d that the exterior surfaces of the filters were becoming progressively discolored. For the SUF samples, the discoloration consisted mainly of gray areas, which appeared to be associated with the location of the U fragments [both DU and DU(Ti)]. For the pH 5 samples, the discoloration pattern was more complex, with gray areas occurring together with yellow and orange areas. These discolorations were most intense in the region of the U fragment, but extended beyond the fragment margin, and in some cases, discolored the filter holder with a yellow deposit as well. This latter deposit was firmly affixed to the plastic. In the same time, very small black grains began to appear within the solvent. The origin of this material is not known. It appears unlikely that visible U particles could have permeated the membrane filters where the pore size was 0.2 μm. Nor is it known whether the black particles were indeed U. However, the presence of this particulate material, if U, would tend to bias the dissolution results upward, as this material was analyzed along with the solvent. Additional information could be obtained by repeating the *in vitro* study and analyzing the particles with appropriate microchemical techniques, e.g., electron energy loss spectrometry or secondary ion mass spectrometry.

When the dissolution study was terminated and the filter holders disassembled, a significant quantity of very fine particulate material was found within the filter "sandwich," along with the remaining U fragment (Figs. 1–4). The surface of the fragment itself was severely corroded, and the edges of the fragment were smoothed down. Both the fragment and the particles were generally black, with a brownish cast also noted in the pH 5 samples. No yellow or orange material was observed within the sample volume contained by the filters. There appeared to be a greater quantity of particles in the pH 5 samples than in the SUF samples.

Figure 1. Photo of DU fragment after 30 days dissolution in SUF solvent.

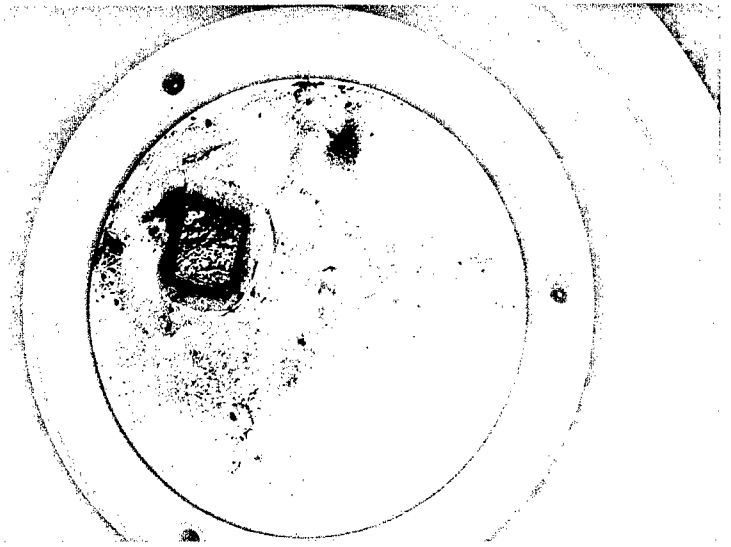

Figure 2. Photo of DU(Ti) fragment after 30 days dissolution in SUF solvent.

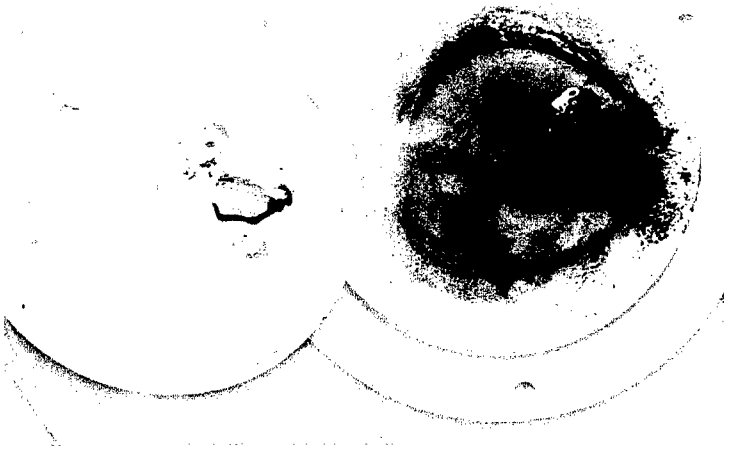

Figure 3. Photo of DU fragment after 30 days dissolution in pH5 solvent.

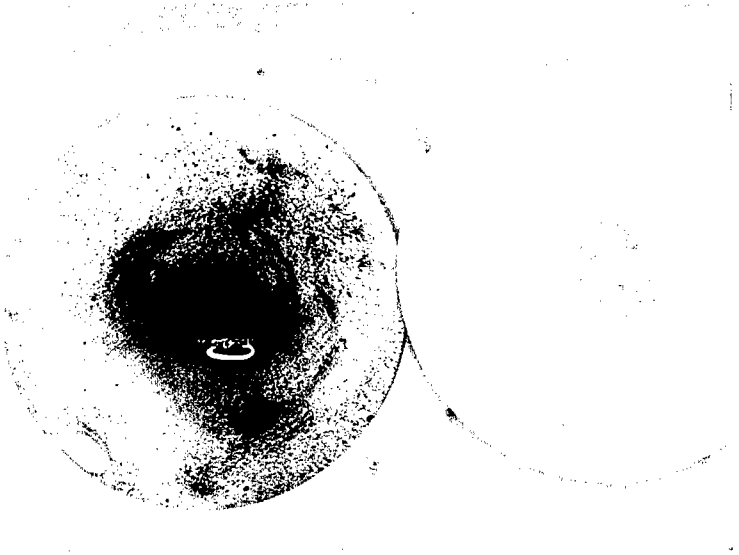

Figure 4. Photo of DU(Ti) fragment after 30 days dissolution in pH5 solvent.

The exact meaning of the observed color changes is not known. However, it is surmised that the various changes are indicative of a multi-step fragmentation-dissolution process whereby U metal is transformed into soluble U ions (UO_2++).

The *in vitro* dissolution data were best fitted to single-component exponential functions. The results of the fitting are summarized in Table 3 and the data and curves illustrated in Figures 5–8. Each curve contains the data from the respective duplicate samples. Two trends are identifiable: 1) both forms of DU were more soluble in pH 5 solvent, and 2) DU was somewhat more soluble in either solvent than DU(Ti).

Table 3

Single-Component Exponential Fit Parameters

for *In Vitro* Dissolution Samples

Material	Solvent	Intercept (%)	Rate Constant (d^{-1})	Half Life (d)
DU	SUF	99.8	0.00425	163
DU(Ti)	SUF	99.9	0.0025	277
DU	pH 5	101.5	0.0113	61
DU(Ti)	pH 5	101	0.0085	81.5

In the first case, the DU and DU(Ti) samples dissolved respectively two and three times more rapidly in pH 5 solvent. Although comparative *in vivo* bioassay data are not available to date, it is expected that the dissolution rates obtained with the SUF solvent will better approximate the *in vivo* rates. This is based on the fact that SUF was originally formulated to mimic the chemical composition of extracellular fluids such as serum. The pH 5 solvent on the other hand was designed to model the intraphagolysosomal pH of alveolar macrophages, and thus would be most appropriate for inhalation exposures. Because the DU fragments were embedded in muscle, it is expected that the chemical milieu will more closely resemble extracellular fluid. Thus, the half-times of 163 d for DU and 277 d for DU(Ti) are believed more relevant.

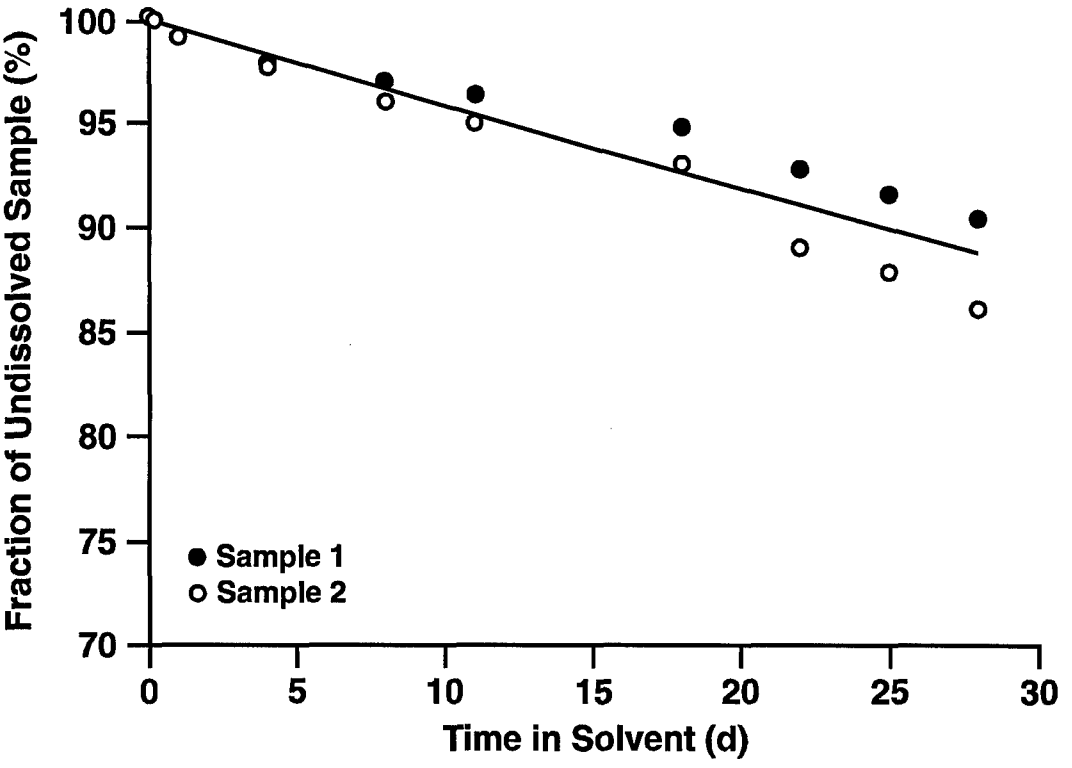

Figure 5. Dissolution of DU in SUF solvent.

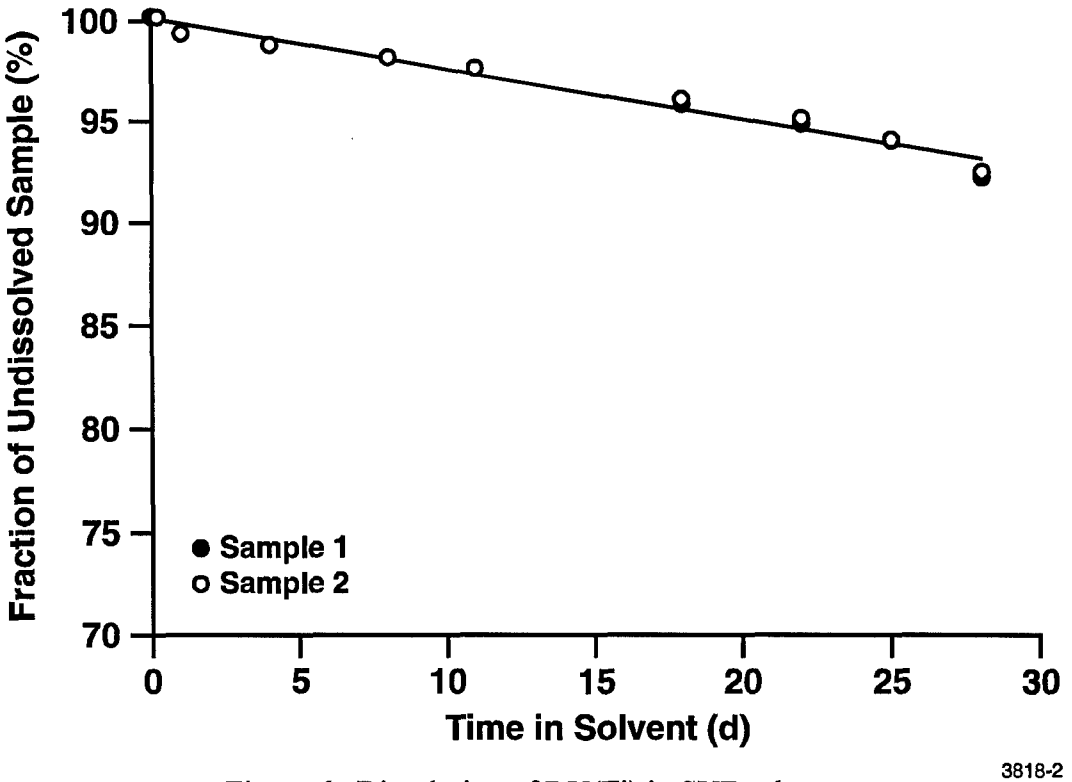

Figure 6. Dissolution of DU(Ti) in SUF solvent.

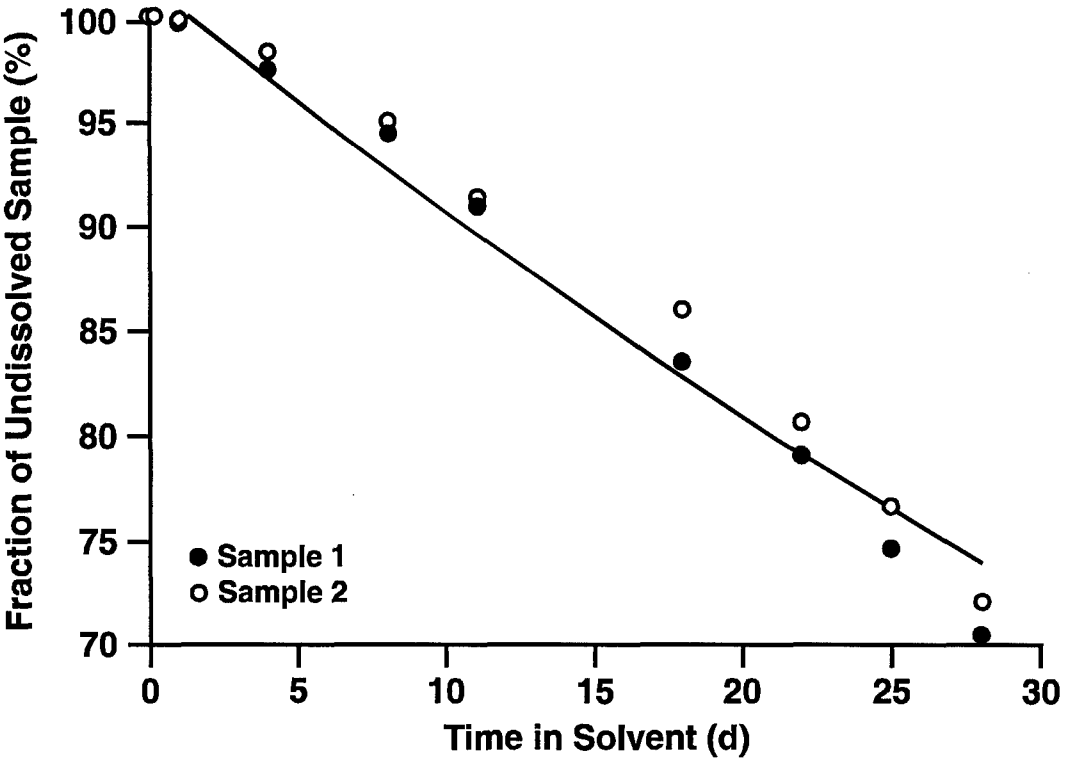

Figure 7. Dissolution of DU in pH 5 solvent.

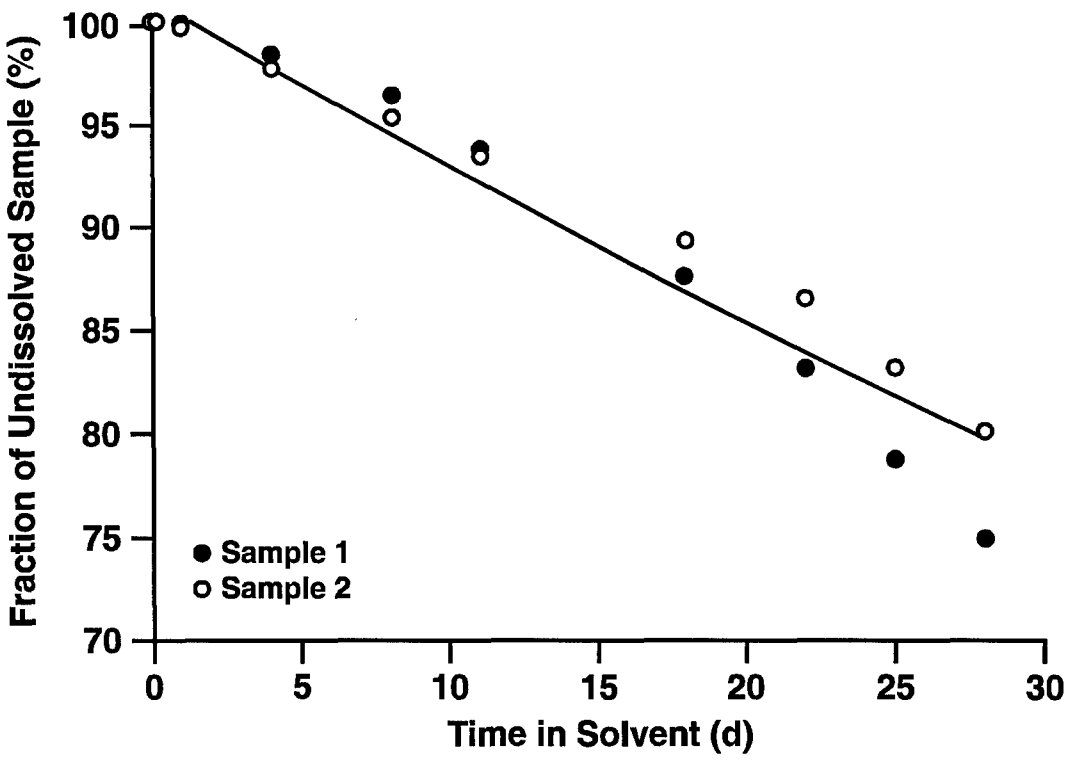

Figure 8. Dissolution of DU(Ti) in pH 5 solvent.

It is interesting to note that the addition of 0.75% Ti to the DU appears to have decreased its solubility in these aqueous test environments. However, the differences in the two solvents, although statistically significant, are less than a factor of two, and do not change one's impression of the intrinsic solubility of the DU metal. In all cases, the solubilities would be described as moderate in the parlance of respiratory tract dosimetry models, i.e., type M absorption class (ICRP, 1994) or class W solubility (ICRP, 1979). There is no comparable dosimetry model for wounds. It remains to be seen whether the *in vivo* data relate to the *in vitro* results.

It is also important to note that the *in vivo* excretion results from the pilot study do not agree with the above *in vitro* data. *In vivo*, more U from DU(Ti) was excreted in the urine, compared to DU. In addition, the levels of U in the kidneys of the DU(Ti) rats and mice were also higher. Both indicate that DU(Ti) was more soluble *in vivo* than *in vitro*. The reasons for these differences are presently not understood.

B. Carcinogenesis Study

A long-term carcinogenesis bioassay study with intramuscularly implanted DU(Ti) pellets and fragments was designed to determine the carcinogenic potential of DU. The DU(Ti) alloy was chosen because it is the same material found in the shrapnel it also behaves somewhat differently in the body compared to DU, as determined by the pilot study. The sizes and shapes of the DU(Ti) used are similar to the range of sizes and shapes of DU(Ti) fragments embedded in soldiers wounded in the Gulf War (Castro *et al.*, 1996). Cylindrical DU(Ti) pellets (2.0 mm long × 1.0 mm in diameter) were obtained from the Manufacturing Sciences Corporation. Two sizes of DU(Ti) fragments (2.5 mm × 2.5 mm × 1.5 mm and 5.0 mm × 5.0 mm × 1.5 mm) were cut from DU(Ti) foil obtained from the Manufacturing Sciences Corporation.

The physical characteristics of the pellets and fragments used in the study are shown in Table 4. The values for individual pellets and fragments are shown as well as those for the total number implanted in each rat. Four implants were made per rat to allow an overlap of physical characteristics among the various groups of rats. For example, the four 2.5 × 2.5 mm fragments have the same mass as one 5.0 × 5.0 mm fragment, and the surface radioactivity of four pellets is similar to that of one 2.5 × 2.5 mm fragment. Using this grid of exposures, we optimize our ability to determine if DU(Ti) is carcinogenic, and, if carcinogenic, to determine the

influence of total U mass and individual implant mass on tumor incidence. We also know the surface area, which is correlated with the surface radioactivity. However, it is less clear how this information can be used.

Table 4

Physical Characteristics of Pellets and Fragments Implanted in Rats

Implant	Number per Rat	Volume[a] (mm^3)	Mass[a] (mg)	Surface Area[a] (mm^2)	E alpha[b] (nCi)
DU(Ti) Pellets[c]	1 pellet	1.6	30	7.9	0.16
"	4 pellets	6.4	119	31.4	0.64
"	20 pellets	31.4	584	157	3.2
DU(Ti) Small Fragments[d]	1 fragment	9.4	175	27.5	0.54
"	4 fragments	37.5	698	110	2.2
DU(Ti) Large Fragments[e]	1 fragment	37.5	698	80	1.6
"	4 fragments	150	2790	320	6.4
DU(Ti) Foil[f]	1 foil	450	8370	705	14
Thorotrast® Injection	1 site	0.057	–	–	3.1

[a]Equations:

	Volume	Surface Area	Mass
Pellet	$V = \frac{\pi D^2 L}{4}$	$S = \pi DL + \frac{\pi D^2}{2}$	$M = V \times \rho$
Fragment	$V = L^2 T$	$S = 2L^2 + 4LT$	

where: D = diameter, L = length, T = thickness, ρ = density of DU(Ti) = 18.6 mg/mm^3.

[b]E alpha = effective alpha-particle activity emanating from the surface of the DU(Ti) in nCi.

[c]Dimensions of DU(Ti) pellets: 1.0 mm in diameter and 2.0 mm in length.

[d]Dimensions of DU(Ti) fragments: 2.5 mm × 2.5 mm × 1.5 mm.

[e]Dimensions of DU(Ti) fragments: 5.0 mm × 5.0 mm × 1.5 mm.

[f]Dimensions of DU(Ti) foils used in pilot study 15 mm × 20 mm × 1.5 mm.

Fragments (5.0 mm × 5.0 mm × 1.1 mm) of Ta obtained from Goodfellow Corp. (Berwyn, PA) are used as a negative control. Thorotrast®, produced by Hyden Chemical Corp, New York, NY, and supplied by Mr. Jim Humphreys, AEA Technology, Harwell, England, is used as a positive carcinogenic control. The distribution, retention, and late effects of ThO_2 used

as a radiographic contrast medium in people have been summarized (Swarm 1967; van Kaick *et al.*, 1995).

The experimental design for the 2-y carcinogenesis study is summarized in Table 5; 358 12-wk-old male Wistar rats (Charles River Laboratories, Wilmington, MA) are used in this study. The Wistar strain was chosen because it is larger than the F344, resulting in a larger muscle mass for implantation. In addition, the Wistar rat does not have a high incidence of nephropathy that could confound the results (Gray, 1986). Survival and tumor incidence data are readily available (Bomhard, 1992; Walsh and Porteracki, 1994).

Table 5

Experimental Design for a 2-Year Carcinogenesis Study of DU(Ti) Pellets and Fragments Intramuscularly Implanted in Male Wistar Rats

Type of Implant	Size (mm)	Number of Implants	Total Number of Rats[a]
DU(Ti) pellets	2.0 × 1.0 dia.	4	50
DU(Ti) fragments	2.5 × 2.5 × 1.5	4	50
DU(Ti) fragments	5.0 × 5.0 × 1.5	4	50[b,c]
DU(Ti) fragments	2.5 × 2.5 × 1.5	4	36[b,c]
Thorotrast® injection	0.050 mL	2	50
Ta fragments	5.0 × 5.0 × 1.1	4	50
Sham implant surgery	NA	0	22[c]
Sham implant surgery	NA	0	50
Total number of rats	–	–	358[d]

[a]Any rats that die within 48 h of the implantation surgery will be replaced. Sixteen rats were ordered as spares. Unused spare rats will be euthanized.

[b]Urine samples to be analyzed for uranium will be collected at selected intervals from six rats from each experimental group. These rats will not be scheduled for serial sacrifice.

[c]Serial sacrifice of rats, dosimetry, hematology, clinical chemistry, and histopathology: four rats at each of the following intervals after implantation of the 5.0 × 5.0 × 1.5 DU(Ti) fragments: 1 week, and 1, 2, 4, 6, and 9 mo. Six rats will be sacrificed at 12 and 18 mo. The eight implant surgery controls that will be treated in parallel with the rats noted in the previous sentence will be sacrificed at 18 mo.

[d]All surviving rats will be sacrificed 2 y after implantation of the metals.

Fifty rats per group are required, except for the sacrifice series groups (Table 5). The group of 50 rats is the standard group size in the National Toxicology Program Statement of Work and the EPA Guidelines 40 CFR 798: 3320 - "Combined Toxicity and Oncogenicity Testing."

The size, mass, and number of implants used are consistent with those used by AFRRI in a 12-mo study in rats, i.e., the surface area of the 5.0 mm × 5.0 mm × 1.5 mm fragment of DU(Ti) is similar to that which resulted in weight loss in the rats with the implanted fragments.

Before implantation surgery, the DU(Ti) pellets, DU(Ti) fragments, and Ta fragments were weighed, then cleaned to remove the oxide formation from the surface of DU metal (Tonry, 1993). Cleaning involved immersion in an industrial detergent, rinsing in absolute ethyl alcohol, sterilization by immersion in a 50% nitric acid solution for 3 min, and rinsing with sterile water. All pieces were stored in acetone to inhibit oxidation prior to implantation. This is the same procedure used in studies at AFRRI (Castro *et al.*, 1996).

Twenty-four-hour urine collections were scheduled for six rats with four 5.0 mm × 5.0 mm × 1.5 mm DU(Ti) fragments and six rats with four 5.0 mm × 5.0 mm × 1.5 mm Ta implants. The urine samples were collected daily for the first 7 d after implantation; twice per week from days 8–28; once per week from days 29–90; and once every 2 wk from day 91 through 2 y (Table 5). Rats with the implanted Ta fragments serve as controls to determine the background level of U in urine samples; 24 h urine samples were also collected from six rats with four implanted DU(Ti) pellets on the same schedule through 90 d (Table 5).

Rats were entered into the study in three blocks of 80 to 85 rats each, one block of 50 and one block of 44 rats. Those in the first three blocks will be observed for 2 y, sacrificed, and examined histologically. Forty-four rats having either 2.5 mm × 2.5 mm × 1.5 mm DU(Ti) fragments implanted or having experienced only sham implant surgery are being serially sacrificed at intervals to 18 mo for dosimetry, hematology, clinical chemistry, and histopathology. Injection of rats with Thorotrast® was originally scheduled concurrently with the implantation of Du(Ti). However, difficulties were encountered in obtaining the Thorotrast® from England, the only supplier available, and these rats were injected about 3 mo after the rats were implanted with DU(Ti). This will delay the completion of the studies to September 30, 1999.

The weight gains for the rats implanted with the larger fragments were significantly less than for the other groups (Fig. 9). The reason for the reduced weight gain is yet to be determined.

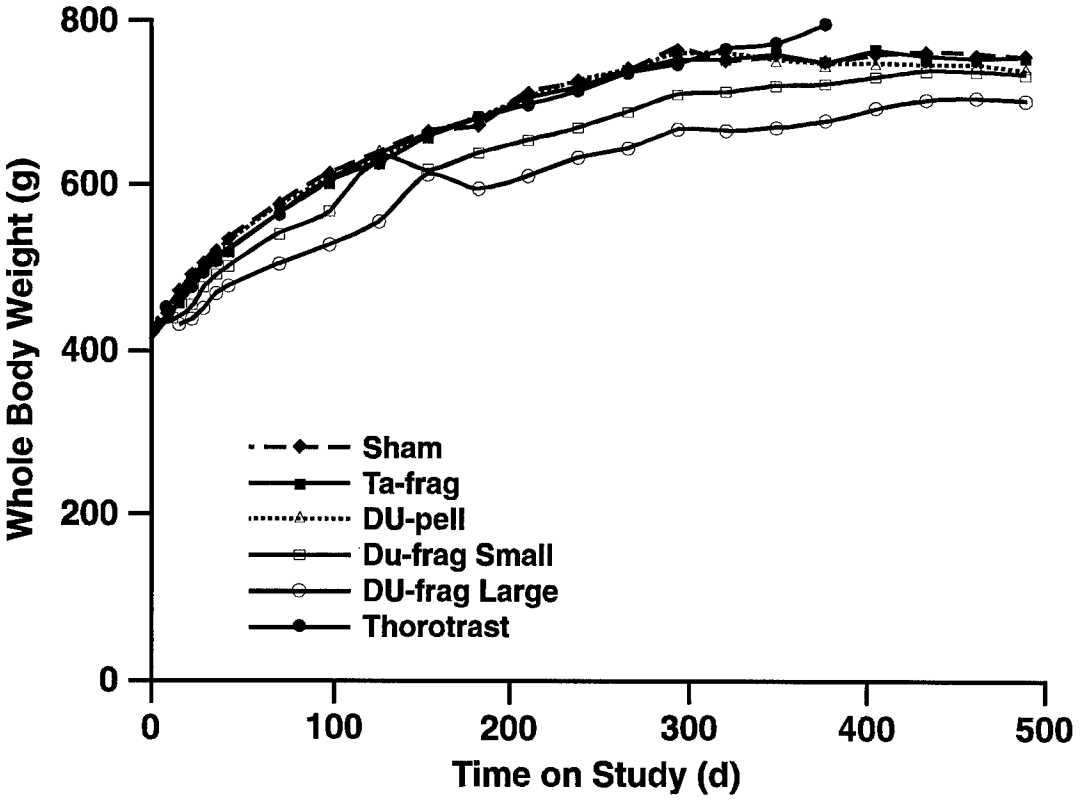

Figure 9. Body weights of rats implanted with various metal fragments. 3818-5

The survival of the various exposure groups as of July 31, 1998, is shown in Table 6. This date is about 15 months after implantation for all groups except the Thorotrast® group, which is about 12 months after injection. All of the groups with DU(Ti) implants have similar survivals that are not significantly different from the survival of the Ta implanted group.

The radiographic appearance of the DU(Ti) fragments changed markedly during the first year after implantation. At implantation, the fragments were smooth with sharp edges (Fig. 10). At 21 days after implantation, small radiographically dense blebs extended from the edges of the fragments (Fig. 11). At 1 y after implantation, the edges of the fragments were rounded and misshapen on the radiographs (Fig. 12). These radiographic changes may indicate a corrosion of the surfaces of the fragments, which was more obvious on the 5.0 mm x 5.0 mm x 1.5 mm size than the smaller fragments.

Table 6

Survival of Rats Implanted with Various Metal Fragments

(as of July 31, 1998)

Group	# Ani/ Group	Died	Moribund Sacrifice	Alive	Scheduled Sacrifice
Sham	50	5	2	43	0
Ta frag	50	8	3	39	0
Thorotrast	50	2	1	47	0
DU pellet	50	6	8	36	0
DU frag 2.5 x 2.5 mm	50	9	5	36	0
DU frag 5.0 x 5.0 mm	50	6	6	38	0
Sham (sacrifice)	22	2	1	4	15
DU frag (sacrifice)	36	2	1	4	29
Total	**358**	**40**	**27**	**247**	**44**

The animals on 2-y study are being observed at least twice daily and moribund or terminally ill animals euthanized. Animals are observed daily in their cages and weighed every other month. All surviving animals will be sacrificed once 90% of any one group has died or at 24 mo, whichever occurs first. Complete necropsies are performed with examination of all organ systems, paying special attention to the implant sites and the urinary system. Histological examination is routinely performed on the implant sites, including site neoplasms, gross lesions that are potential metastases, and the kidneys. Neoplasms at the implant sites will be characterized with light microscopy and immunohistochemistry. Ultrastructural studies of the tumor cells have implicated a pluripotential mesenchymal cell type possessing morphologic characteristics consistent with cell types of the microvasculature as the preneoplastic parent cell (Johnson *et al.*, 1973). Thus, cell identifications will focus on endothelial cells, smooth muscle cells, and pericytes.

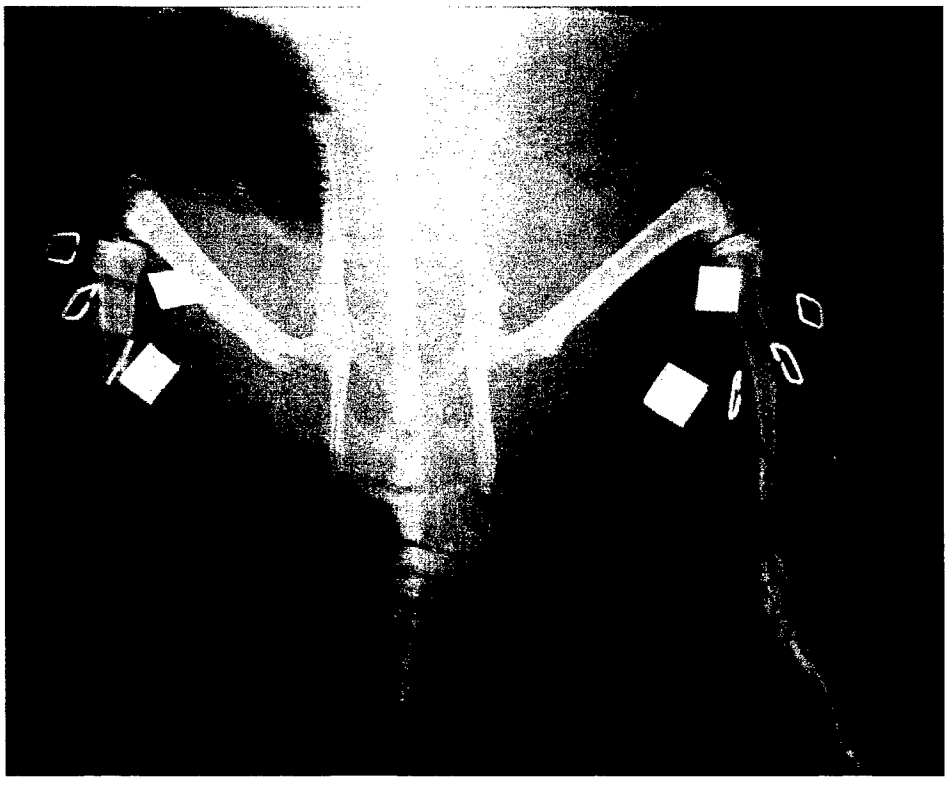

Figure 10. Radiograph of 5.0 x 5.0 x 1.5 mm DU(Ti) fragments on day of implantation.

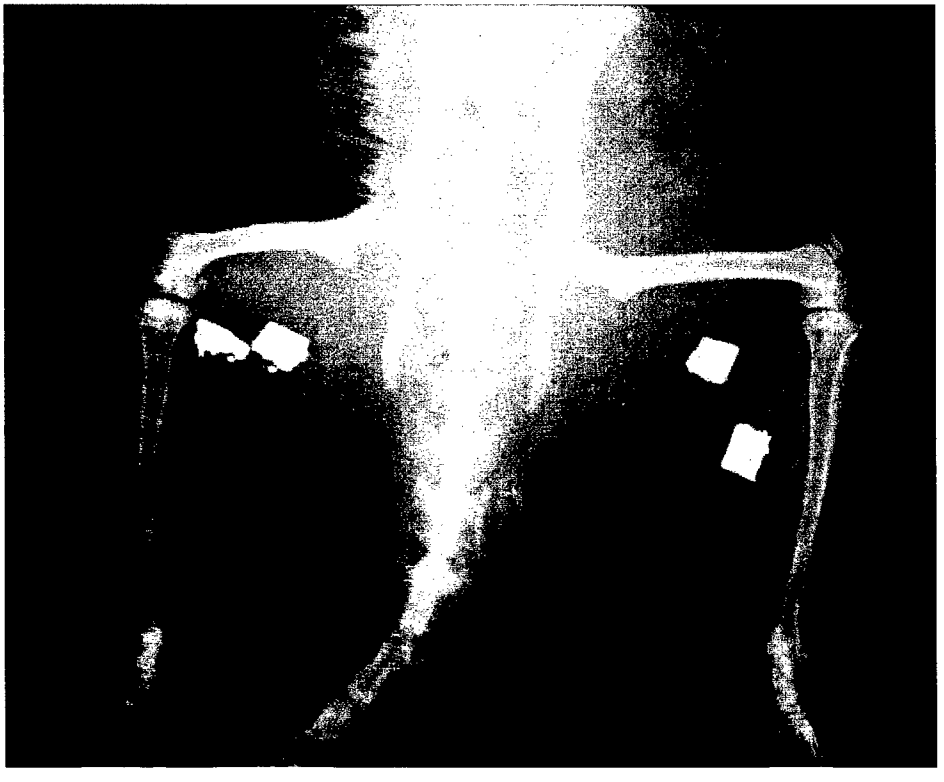

Figure 11. Radiograph of 5.0 x 5.0 x 1.5 mm DU(Ti) fragments 3 weeks after implantation.

Figure 12. Radiograph of 5.0 x 5.0 x 1.5 mm DU(Ti) fragments 1 year after implantation.

When compared with the foreign-body carcinogenesis model, this bioassay protocol uses materials and includes observations that mimic what is seen in the exposure of the Gulf War veterans to DU-containing shrapnel. The bioassay protocol is a simple, straightforward approach to determining the carcinogenic potential of DU(Ti).

III. CONCLUSIONS

We have demonstrated that the subcutaneous foreign-body carcinogenesis system described by Brand *et al.* (1975) is not appropriate to study the carcinogenesis of implanted DU(Ti) fragments in rats. An alternate method, using a carcinogenesis bioassay protocol, is being used to study the carcinogenic effects of implanted DU(Ti) fragments. These studies will be finished in September 1999. A no-cost extension of the contract period has been obtained.

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V. APPENDIX

A. Acronym and Symbol Definition

AFFRI	=	Armed Forces Radiobiology Research Institute
CPH	=	Cox proportional hazard
DU	=	Depleted uranium
DU(Ti)	=	Depleted uranium + 0.75% titanium
LRRI		Lovelace Respiratory Research Institute
SUF	=	Synthetic serum ultrafiltrate
Ta	=	Tantalum
U	=	Uranium

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
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